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Authors	Bruno_J_G Cornette_J_C
Journal title	Microchemical Journal
ISSN	0026-265X
Publisher	Academic Press USA
Year of publication	1997
Volume	56
Issue	3
Supplement	0
Page range	305-314
Number of pages	10
User name	Adonis
Cost centre	Development
PCC	\$30.00
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## An Electrochemiluminescence Assay Based on the Interaction of Diaminotoluene Isomers with Gold(I) and Copper(II) Ions

John G. Bruno\*<sup>1</sup> and Jimmy C. Cornette<sup>†</sup>

\*Applied Research Associates, 139 Barnes Drive, Suite 2, Tyndall Air Force Base, Florida 32403;  
and <sup>†</sup>U.S. Air Force, Armstrong Laboratory, Environics Directorate, 139 Barnes Drive,  
Suite 2, Tyndall Air Force Base, Florida 32403

Received April 12, 1996; accepted July 19, 1996

A novel assay for the determination of 2,4- and 3,4-diaminotoluene (DAT) isomers based on the low-level electrochemiluminescence (ECL) reaction of these molecules with the group IB transition metal ions  $\text{Au}^+$  and  $\text{Cu}^{+2}$ , respectively, in aqueous solution is described. DAT isomers were screened for ECL against a repertoire of 32 metals, including metal ions such as  $\text{Cu}^{+2}$ ,  $\text{Eu}^{+3}$ ,  $\text{Mg}^{+2}$ ,  $\text{Ru}^{+3}$ , and  $\text{Tb}^{+3}$  associated with other known ECL complexes, at 1:3 added metal ion:ligand molar ratios. The 1:3 molar ratio presumed tris-bidentate octahedral metal coordination complex formation, which generally yielded optimal ECL intensity. The apparent specificity of  $\text{Au}^+$  for 2,4-DAT and  $\text{Cu}^{+2}$  for 3,4-DAT, as indicated by ECL measurements, may be partly based on ionic size as  $\text{Au}^+$  has nearly twice the ionic diameter of  $\text{Cu}^{+2}$  and thus may form a coordination complex with the *meta*, but not the *ortho* DAT. Other DAT isomers were screened and exhibited mildly enhanced ECL with various metal ions, including group IB transition metal ions, but these ECL enhancements were not statistically significant. In some cases, titration of DAT ligands with  $\text{Cu}^{+2}$  and  $\text{Au}^+$  over broad concentration ranges produced nonlinear ECL response curves. Despite low-level ECL, sensitivities in the ppm range for  $\text{Au}^+$ ,  $\text{Cu}^{+2}$ , and their respective DAT isomers were achieved. Time dependence was observed for some of the ECL reactions, including the  $\text{Ru(III)}$ -bipyridine model system, in which the ECL intensity grew markedly over several hours. No ECL enhancements over background were observed with two dinitrotoluene isomers or an aminonitrotoluene screened against the same set of 32 metals. This novel ECL approach may have applications in the determination of some aminoaromatics from degradation of explosives (e.g., TNT) as well as detection and quantitation of various transition metals in industrial wastewater streams and groundwater supplies. In terms of fundamental science, the present data are probably of interest as an example of size-dependent molecular recognition of metal ions which can be detected by ECL. © 1997 Academic Press

### INTRODUCTION

Trinitrotoluene (TNT), other nitroaromatics, and their byproducts, or degradation products, especially the aminoaromatics, are significant military and industrial pollutants in terms of their toxicity, distribution, and relative abundance (1) and are consequently of environmental concern. Likewise, many of the heavy and transition metals are highly toxic industrial pollutants. Assays and environmental sensors for toxic organics, such as TNT (2) and metals, are continuously being developed and improved to yield better sensitivity and reliability for use in complex matrices as well as design simplicity and assay execution under field conditions.

Chemiluminescent (CL) assays have demonstrated extreme sensitivity, which in some cases rivals that of radioisotopic methods (3–10). Electrochemiluminescence

<sup>1</sup> To whom correspondence should be addressed.

(ECL) is a form of chemiluminescence which involves induction of luminescence via application of a voltage potential (3-14). ECL has already been applied to such diverse problems as sensitive detection of biotoxins (6), nucleic acids (7), and bacterial pathogens (6, 8). ECL has a distinct advantage over other forms of CL in that the luminescence can be controlled by the applied voltage at the working electrode (3-5).

ECL molecules can be entirely organic, organic-metal ion coordination complexes, or other types of molecules (3). Ruthenium trisbipyridine ( $\text{Ru}(\text{bpy})_3$ ) is, perhaps, the best known of the organic-metal ion coordination complex ECL molecules (3-10), although other transition metal complexes involving Cr, Cu, Eu, Ir, Mg, Os, and Tb have been observed to exhibit ECL under appropriate conditions (3, 11-14). ECL can occur by various chemical mechanisms (3).  $\text{Ru}(\text{bpy})_3$ -based ECL resembles fluorescence and phosphorescence in that an excited electronic state is induced in the ECL molecule which subsequently relaxes to a ground state to yield light. In the model  $\text{Ru}(\text{bpy})_3^{+2}$  ECL system,  $\text{Ru}(\text{bpy})_3^{+2}$  is oxidized to the +3 state at the surface of an anode (3-5). Subsequently,  $\text{Ru}(\text{bpy})_3^{+3}$  is placed in an excited state ( $\text{Ru}(\text{bpy})_3^{+2*}$ ) by a high energy free radical electron transfer from an electron carrier such as nascent TPA $^{\cdot\cdot}$  radical, which is generated simultaneously at the anode. Relaxation of the high energy  $\text{Ru}(\text{bpy})_3^{+2*}$  to the ground state yields luminescence at approximately 620 nm (3-8).

ECL is essentially a surface redox reaction which allows ECL molecules, such as  $\text{Ru}(\text{bpy})_3^{+2}$ , primarily near the surface of the anode to undergo the ECL redox reaction sequence (3-5, 9). This is an advantage in immunomagnetic bead-ECL assays as the overlying fluid phase contributes only negligible ECL compared to the magnetically captured antigen-antibody-ECL tag complexes on the anode surface (6-8), thus obviating "wash" steps. However, the surface redox phenomenon is a disadvantage for "solution phase" ECL measurements as sensitivity may be somewhat compromised. Thus, the use of immobilized ECL complexes, while not completely necessary, is probably desirable.

The concept that specific metal ions can be captured by particular organic ligands to form ECL compounds was used in the present work as the theoretical basis for investigation of several diaminotoluene (DAT)-metal ion ECL reactions. While DATs are not direct biodegradation products of TNT, DATs are biodegradation products of dinitrotoluene (DNT) which is present in TNT and DATs are related to other compounds that are TNT breakdown products (1). Therefore, the ECL reactions described herein may form the basis for sensors to be used for environmental detection and analysis of TNT biodegradation.

## MATERIALS AND METHODS

### *Metal Ions, Ligands, and Other Reagents*

Thirty-two atomic absorption standards, consisting of hydrochloric or nitric acid-solubilized metal ions or salts were obtained from Fisher Scientific Co. (Pittsburgh, PA) and SPEX Corp. (Edison, NJ). The metal oxidation states of the 32 standards are given in Table 1. All diaminotoluene isomers (2,3-DAT, 2,4-DAT, 2,5-DAT, 2,6-DAT, and 3,4-DAT), dinitrotoluene isomers (2,4-DNT and 2,6-DNT), 4-amino-3-nitrotoluene (4-A,3-NT), and 98% tripropylamine (TPA) were obtained from Aldrich

TABLE 1  
Oxidation States of Dissolved Metals Used to Screen Aromatic  
Ligands for Electrochemiluminescence

Metal	Oxidation State	Metal	Oxidation State
Ag	+1	Mg	+2
Al	+3	Mn	+2
As	+3	Na	+1
Au	+1	Ni	+2
Ba	+2	Pb	+2
Ca	+2	Re	+3
Cd	+2	Ru	+3
Co	+2	Sb	+2
Cr	+6	Se	+4
Cu	+2	Sn	+2
Eu	+3	Sr	+2
Fe	+3	Tb	+3
Ga	+3	Tl	+1
Hg	+2	W	+4
K	+1	V	+5
Li	+1	Zn	+2

Chemical Co. (Milwaukee, WI). Bipyridine (2,2'-bipyridine) was obtained from Sigma Chemical Co. (St. Louis, MO). ECL assay buffer was made from 0.15 M  $\text{Na}_2\text{HPO}_4$ , 0.5% Triton X-100, and 0.3 M TPA in 18 MegaOhm deionized water. Cell cleaner buffer was acquired from IGEN Corp. (Gaithersburg, MD).

#### *ECL Assay Preparation and ECL Measurements*

An ORIGEN Analyzer (ECL sensor) was acquired from IGEN Corp. All aromatic ligands were dissolved at 1 mg/ml in absolute methanol. The only exception was 2,5-DAT, which did not completely dissolve in methanol at 1 mg/ml and was dissolved at 0.5 mg/ml and used at double the typical volume of other DAT isomers. For preliminary ECL screening of the 32 metal ions with aromatic ligands, 200  $\mu\text{l}$  (400  $\mu\text{l}$  of 2,5-DAT) of 1 mg/ml ligand solutions were added to an appropriate volume of each individual metal ion standard to produce a 1:3 metal ion:ligand molar ratio in 12  $\times$  75 mm borosilicate glass tubes. The mixtures were gently agitated and allowed to stand at room temperature (RT) for a minimum of 15 min. Sufficient ECL assay buffer (containing 0.3 M TPA) was added to bring the assay volume to 1 ml so that a maximum of 4 measurements of 225  $\mu\text{l}$  each could be made from each tube. In addition, background control tubes consisting of the ligand without any added metal ions, each of the metal ions without ligand, and ECL assay buffer alone were assayed. Total assay set up time was approximately 30 min and final assay pH varied between 7.5 and 8.0 depending on the metal ions used. Tubes were vortex mixed on the ORIGEN Analyzer at 100 rpm and ECL measurements were made for 0.6 s using the default preoperative potential of 0 V and ramping to 2.8 V at a rate of 4.8 V/s with the ORIGEN Analyzer at an assay gain of 1000. Background ECL from 0.3 M TPA (ECL assay buffer) blanks was automatically measured and subtracted from each experimental measurement by the ORIGEN Analyzer. Timed ECL experiments were

conducted in some cases and times are reported as time from the start of the ECL measurement cycle for a given carousel of tubes minus the assay set up time.

Following initial screening with each of the available metal ions for a given ligand, titrations were conducted with metal ions which had previously demonstrated a statistically significant ECL elevation (Student's *t* test,  $\alpha = 0.05$ ) over background ECL levels (i.e.,  $\text{Cu}^{+2}$  with 3,4-DAT and  $\text{Au}^+$  with 2,4-DAT). Two types of titrations were performed; one involving variation of the ligand concentration with the metal ion fixed at the level used for screening and a second involving variation of the metal ion concentration with the ligand concentration fixed at the level used for screening.

## RESULTS

Dinitrotoluene isomers (2,4- and 2,6-DNT) and 4-amino-3-nitrotoluene were screened with the 32 available metal ions (Table 1), which included metals such as  $\text{Cu}^{+2}$ ,  $\text{Eu}^{+3}$ ,  $\text{Ru}^{+3}$ , and  $\text{Tb}^{+3}$  (3, 11–14) that are part of other known ECL compounds. The compounds were screened in 1:3 metal ion:ligand molar ratios by design to favor the formation of octahedral coordination complexes. The DNT and aminonitrotoluene–metal ion data demonstrated that the nitro functional group disallowed or severely inhibited ECL with all metal ions examined.

Conversely, amino functional groups appeared to interact with certain metal ions to produce noteworthy ECL reactions. In particular, 2,3-, 2,4-, 2,5-, 2,6-, and 3,4-DAT were screened against the collection of 32 metal ions. Control solutions of metal ions without ligands, but with TPA, gave no detectable ECL, while some solitary DAT ligand solutions produced appreciable background ECL in the presence of TPA. Preliminary screening experiments demonstrated that, while some low level of ECL enhancement over background was observed for virtually all the DAT isomers, except 2,6-DAT, due to the presence of added transition metal ions, the most intense ECL responses came from the group IB transition metal ions ( $\text{Cu}^{+2}$ ,  $\text{Ag}^+$ , and  $\text{Au}^+$ ) with the 2,4- and 3,4-DATs as illustrated in Fig. 1. Moreover, it appeared that the 3,4-DAT could only produce statistically significant (by Student's *t* test,  $\alpha = 0.05$ ) ECL enhancement with the smaller diameter  $\text{Cu}^{+2}$  ion (1.74 Å ionic diameter; 15), while the 2,4-DAT only produced statistically significant ECL with the larger  $\text{Au}^+$  ion (3.02 Å diameter; 15). The intermediate sized  $\text{Ag}^+$  was of borderline significance with 2,4-DAT and gave no significant ECL with 3,4-DAT (Fig. 1). In terms of coordination chemistry theory, this observed pattern of interaction suggested that the greater spacing between amino groups on *meta*-(2,4-DAT) allowed the formation of a 90° coordination complex with the larger  $\text{Au}^+$  ion, while the shorter distance between amino groups in the *ortho*-(3,4-DAT) isomer prevented octahedral coordination complex formation with the larger  $\text{Au}^+$  ion, but allowed complex formation with the smaller  $\text{Cu}^{+2}$  in either octahedral or square planar motifs.

The  $\text{Cu}^{+2}$ –3,4-DAT reaction rapidly produced a light brown solution from the light blue cupric ion solution and the very light orange 3,4-DAT solution in a 1:3 metal:ligand molar ratio. Similarly, a 1:3 molecular ratio of the light yellow  $\text{Au}^+$  solution with the colorless 2,4-DAT produced a dark brown solution.

The well-studied Ru–bipyridine was used as a model system for preliminary investigations of the behavior of metal ion–ligand solution phase ECL titrations and kinetics. Figure 2 illustrates a parabolic titration between  $\text{Ru}^{+3}$  and 2,2'-bipyridine that peaked and declined at higher  $\text{Ru}^{+3}$  concentrations. The octahedral (i.e., 1:3 metal ion:ligand

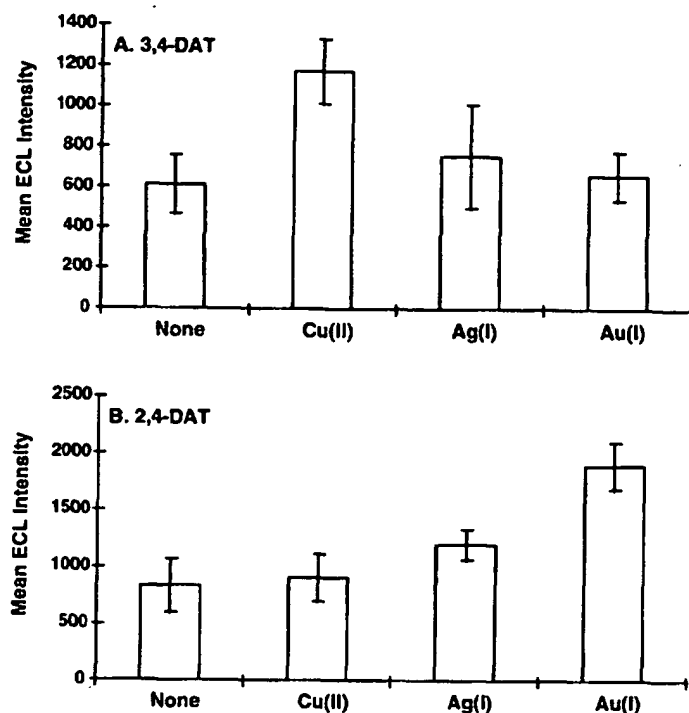


FIG. 1. ECL assessment of group IB transition metal ion interactions with diaminotoluene (DAT) isomers in 1:3 metal ion:ligand molar ratios. The means and  $2 \times$  standard deviations of five independent ECL measurements are depicted. The bars for "none" are controls in which no metal ion was added and indicate the background ECL of the respective DAT isomers.

molar ratio)  $\text{Ru}(\text{bpy})_3$  coordination complex is known to be a strong ECL compound (4, 5, 9, 10) as illustrated by the approximate peak ECL in Fig. 2. It should be emphasized that the molar ratios given merely indicate the molar amounts of bipyridine added in relation to the amount of  $\text{Ru}^{+3}$  present in solution and that this may be a very different situation from crystallizing out true  $\text{Ru}(\text{bpy})_3$  for subsequent solvation and ECL assessment. Figure 2 also indicates a pronounced time dependence for the  $\text{Ru}^{+3}$ -bipyridine reaction. In general, it appears from the figure that ECL measurements taken in the first hour were fairly well clustered together for each titration point on the ECL response curve, but by 3 h, the ECL had increased dramatically across the range of  $\text{Ru}^{+3}$  concentrations examined.

An ECL intensity time dependence was also observed in the  $\text{Cu}^{+2}$ -3,4-DAT system (Fig. 3) even for measurements taken within the first 30 min. Figure 3 demonstrates an overall ECL intensity of  $\text{Cu}^{+2}$  with 3,4-DAT that is at least an order of magnitude less intense than is evident in the  $\text{Ru}^{+3}$ -bipyridine system over a comparable time interval. However, the figure depicts a very linear ECL response of  $\text{Cu}^{+2}$  to widely varied concentrations of 3,4-DAT from 1 to 200 ppm. The figure also indicates an ECL assay lower detection limit on the order of  $\leq 1$  ppm of 3,4-DAT when reacted with a fixed concentration of 32 ppm of  $\text{Cu}^{+2}$  (selected from the 1:3 metal ion:ligand preliminary screen). The detection limit of  $\leq 1$  ppm was feasible due to the extremely

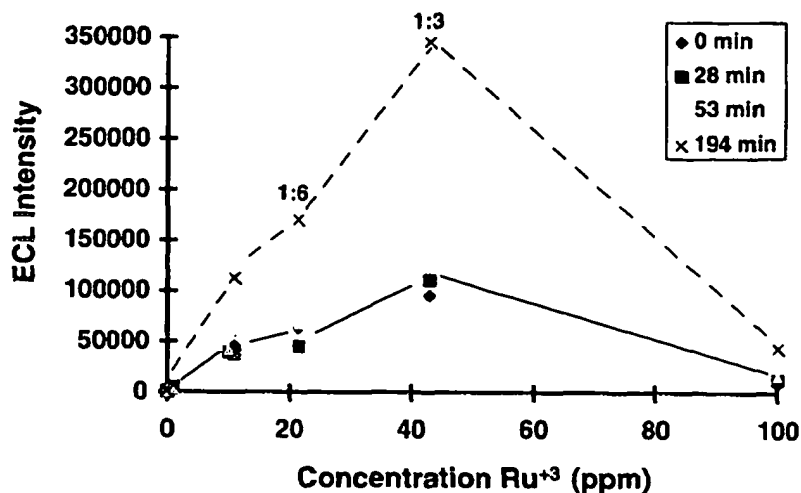


FIG. 2. ECL response of a Ru(III)-bipyridine solution phase model system (fixed 2,2'-Bipyridine: 220 ppm). Times are given as time from the start of the first ECL measurement cycle. Ratios above certain data points are indicative of the added metal ion to ligand molar ratios.

low background ECL (essentially zero) from solutions of  $\text{Cu}^{+2}$  in ECL assay buffer without 3,4-DAT.

However, when the ligand (3,4-DAT) concentration is held constant at 200 ppm and the  $\text{Cu}^{+2}$  concentration is varied, the sensitivity of the ECL reaction to  $\text{Cu}^{+2}$  is compromised somewhat by the background ECL from 3,4-DAT as evident at the zero added  $\text{Cu}^{+2}$  data point in Fig. 4. Figure 4, like Fig. 3, demonstrates an ECL time dependence for the  $\text{Cu}^{+2}$ -3,4-DAT reaction and suggests that a 1:6 or 1:3 metal ion:ligand molar ratio was generally optimal for this reaction system as the ECL

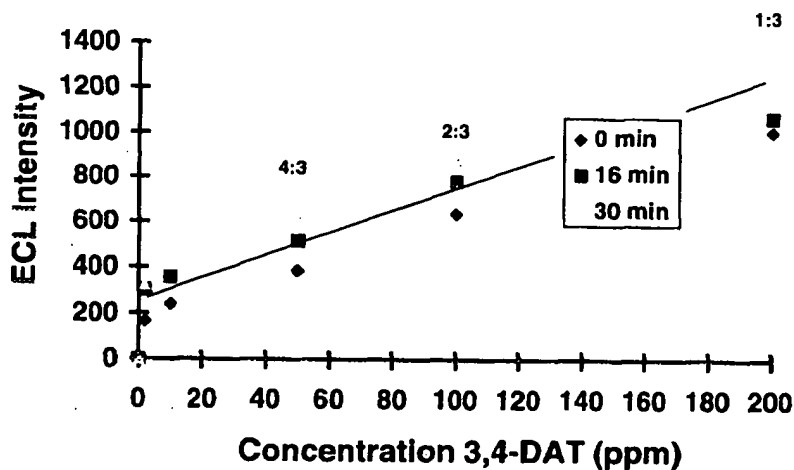


FIG. 3. Linear ECL response of Cu(II) to varied concentrations of 3,4-DAT (fixed Cu(II): 32 ppm).



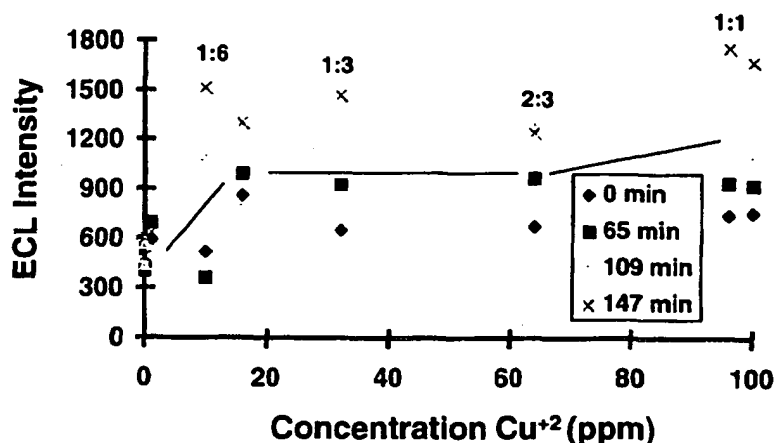


FIG. 4. ECL response of 3,4-DAT to varied concentrations of Cu(II) (fixed 3,4-DAT: 200 ppm).

response peaked at the 1:6 to 1:3 ratio and generally leveled off with ligand concentrations beyond these points.

Similar results were obtained for the Au<sup>+</sup>-2,4-DAT ECL titrations in that the best molar ratio for ECL appeared to be 1:3 metal ion:ligand as evidenced by Figs. 5 and 6. Again, the 1:6 metal:ligand molar ratio was comparable in ECL intensity to the 1:3 ratio (Fig. 6). Unlike the Cu<sup>2+</sup>-3,4-DAT assay, the Au<sup>+</sup>-2,4-DAT titration demonstrated no time dependence and did not appear to have a broad linear response range (Figs. 5 and 6). Another dissimilarity to the Cu<sup>2+</sup>-3,4-DAT reaction was that at the 2:3 Au<sup>+</sup>:2,4-DAT molecular ratio, the ECL response was completely inhibited (Fig. 6). This resembles the Ru<sup>3+</sup>-bipyridine result shown in Fig. 2 at 100 ppm Ru<sup>3+</sup> and may be related to the usurping of free radical electrons from TPA<sup>••</sup> (4, 5) by unchelated Au<sup>+</sup> or Ru<sup>3+</sup> ions, which are relatively strong oxidizers. Interference in the high energy

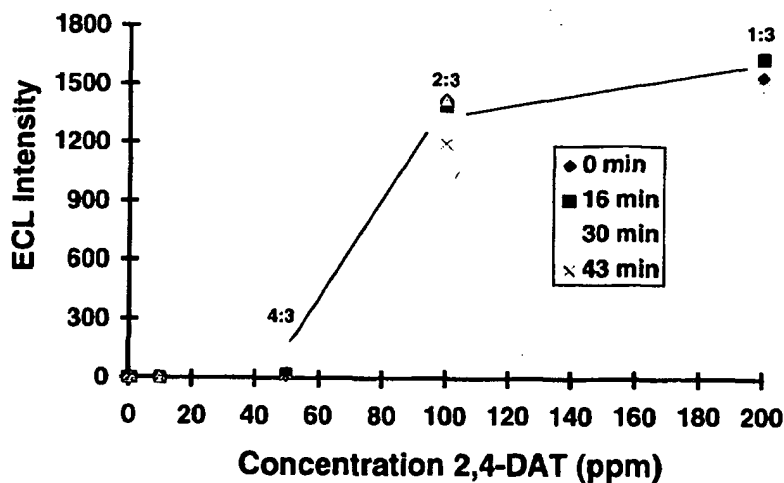


FIG. 5. ECL response of Au(I) to varied concentrations of 2,4-DAT (fixed Au(I): 99 ppm).

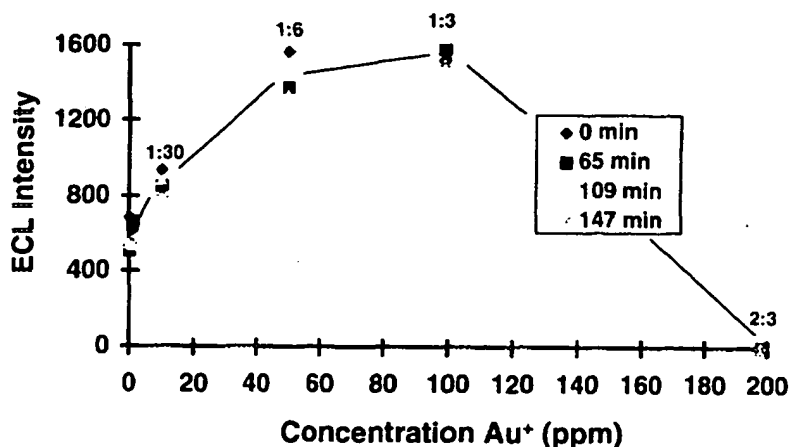


FIG. 6. ECL response of 2,4-DAT to varied concentrations of Au(I) (fixed 2,4-DAT: 200 ppm).

free radical electron transfer from TPA<sup>••</sup> to the ECL compound would inhibit or prohibit ECL.

### DISCUSSION

The data presented here do not definitively rule out the possibility of DAT polymers, oxidation products, or other unknown ECL compounds as the source of the observed ECL produced by the addition of group IB transition metals to solutions of DAT isomers. Indeed, color changes such as the conversions of 2,4-DAT and 3,4-DAT solutions from clear or lightly colored to brownish suggest polymerization or monomer oxidation in the presence of metal ions. However, the metal ion size correlations with the spacing of amino groups on DATs to produce significant ECL increases speak strongly in favor of the formation of coordination complexes and a primitive form of size-dependent "molecular recognition." Characterization of the actual metal-organic reaction products is the subject of future research. At present, however, several empirically useful ECL assays have been described, which may find analytical, industrial, or military applications.

The ECL reactions presented here are novel, but similar to other known assays such as the colorimetric and fluorescence assays involving chelation of Se<sup>4+</sup> with 2,3-diaminonaphthalene (16) or 3,3'-benzidine (17). The presently described ECL assays are potentially useful in several ways. First, the assays may be used to detect and quantify DATs, or possibly triaminotoluenes, which are biological breakdown products of explosives (TNT) contamination and are consequently of military and industrial importance (1). Second, the very same reactions may be employed to determine the presence and quantity of some group IB transition metals of environmental and industrial significance.

Several results of fundamental scientific interest were also elucidated by the present study. First, of all 32 metal ions examined, only members of the group IB transition metals yielded statistically significant ECL enhancement in conjunction with any of the DAT isomers. Second, among the ECL-relevant DAT isomers, there was a correlation between size of the metal ion and spacing of the amino groups on the DATs, suggesting

that ECL may be used, in some rare cases, as a means to measure atomic or intramolecular distances or angles. Curiously, some combinations of metal ions and DAT isomers, such as  $\text{Cu}^{+2}$  and 2,3-DAT, which the present hypothesis would predict to be a significant ECL combination, were not. Thus, more must be understood about the effects of amino positioning on the aromatic ring, effects of steric hindrance, and overall molecular orbital theory of these and related compounds.

Dong and Martin (18) have recently reported on similar studies of ECL-based detection which utilize derivatized pyridines and aminoquinolines produced by enzymatic cleavage. In these systems, the cleaved pyridine and aminoquinoline derivatives act as the third ligand for previously crystallized and purified  $\text{Ru}(\text{bpy})_3$ , which is relatively nonelectroluminescent until the third (enzymatically cleaved) ligand becomes tightly bound by heating at  $65^\circ\text{C}$  for 1 h. Dong and Martin made ECL comparisons of various derivatized pyridines and aminoquinolines which showed some general structural trends among the ECL emitters and expressed a desire for strong ECL reactions between metal ions and organic ligands that did not require extensive heating and could therefore be monitored in near real time (18). Clearly, it would also be desirable to avoid significant heating in an environmental field assay or sensor. Thus, other means of enhancing the metal ion-DAT ECL, such as DAT immobilization on electrodes via spacer "arms" to allow three-dimensional coordination complex formation, must be explored.

It should be emphasized as well that only simple ECL intensity measurements were made in the present work and that much more valuable information may be contained in other luminescence emission parameters such as ECL spectra or lifetimes that may reveal more subtle aspects of the metal ion-ligand coordination complex formation.

Of further significance for the current work was the observation of ECL intensity as a function of molar ratios of metal ions to ligands. The model  $\text{Ru}(\text{bpy})_3$  system behaved as predicted in that the 1:3 molar ratio of metal ion to ligand exhibited the most intense ECL even without crystallization or heating. For the  $\text{Au}^+$  and  $\text{Cu}^{+2}$ -DAT assays, a 1:6 or 1:3 added molar ratio generally yielded maximal ECL intensity, suggesting predominate mono- and bidentate octahedral coordination complex formation. In the case of 1:6 metal ion:DAT complexes, on average only one of the DAT amino groups forms a coordinate bond with the central metal ion.

Other interesting aspects of the present study included the facts that nitro groups disallowed ECL enhancement as did the intervening methyl group of 2,6-DAT, presumably because of steric hindrance. Also, noteworthy was the observation that higher concentrations of strongly oxidizing metal ions can severely or completely inhibit the ECL reaction. This may be due to capture of the  $\text{TPA}^{+}$  free radical electron by unbound metal ions, which prohibits excitation of the ECL complex and consequently prevents ECL.

The present assays have lower detection limits or sensitivities in the ppm range for both the metal ions and organic ligands involved. While this is not immediately impressive, it should again be noted that only solution phase measurements were made. The preferred mode of ECL measurement is to use ECL compounds concentrated on the electrode surface to take advantage of the stronger electric field proximal to the electrode (4, 5, 9). It would be reasonable to expect the present ECL assay sensitivities to improve an order of magnitude or more with optimal immobilization of ligands or metal ions on an electrode surface (9).

Overall, the novel ECL reactions described here hold the promise of simple, rapid, and inexpensive environmental assays and sensors. These and similar ECL reactions might be accomplished by miniaturized, low power consumption sensors. As research tools, the present assays and other ECL reactions awaiting discovery may be of utility in determining distances on an atomic scale, studying molecular electronic states, and in characterizing coordinate bonds. The central question of why certain metal ions are efficient ECL participants in specific coordination complexes, while others are not, should be a driving force for basic research and may yield many useful applications of ECL technology. Regardless of our academic understanding of ECL mechanics, useful ECL assays, such as those presented here, will likely continue to emerge and be exploited.

### ACKNOWLEDGMENTS

The authors thank IGEN Corp. for loan of the ORIGIN Analyzer ECL sensor and Dr. Allen J. Bard of the Department of Chemistry and Biochemistry at the University of Texas at Austin for examination of the manuscript.

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AN 1997:471738 CAPLUS  
DN 127:214382

TI An electrochemiluminescence assay based on the interaction of  
diaminotoluene isomers with gold(I) and copper(II) ions

AU Bruno, John G.; Cornette, Jimmy C.

CS Applied Research Associates, Tyndall Air Force Base, FL, 32403, USA

SO Microchemical Journal (1997), 56(3), 305-314 July 1997

CODEN: MICJAN; ISSN: 0026-265X

PB Academic

DT Journal

LA English

CC 80-6 (Organic Analytical Chemistry)

Section cross-reference(s): 50, 61

AB A novel assay for the detn. of 2,4- and 3,4-diaminotoluene (DAT) isomers based on the low-level electrochemiluminescence (ECL) reaction of these mols. with the group IB transition metal ions Au<sup>+</sup> and Cu<sup>2+</sup>, resp., in aq. soln. is described. DAT isomers were screened for ECL against a repertoire of 32 metals, including metal ions such as Cu<sup>2+</sup>, Eu<sup>3+</sup>, Mg<sup>2+</sup>, Ru<sup>3+</sup>, and Tb<sup>3+</sup> assocd. with other known ECL complexes, at 1:3 added metal ion:ligand molar ratios. The 1:3 molar ratio presumed tris-bidentate octahedral metal coordination complex formation, which generally yielded optimal ECL intensity. The apparent specificity of Au<sup>+</sup> for 2,4-DAT and Cu<sup>2+</sup> for 3,4-DAT, as indicated by ECL measurements, may be partly based on ionic size as Au<sup>+</sup> has nearly twice the ionic diam. of Cu<sup>2+</sup> and thus may form a coordination complex with the meta, but not the ortho DAT. Other DAT isomers were screened and exhibited mildly enhanced ECL with various metal ions, including Group IB transition metal ions, but these ECL enhancements were not statistically significant. In some cases, titrn. of DAT ligands with Cu<sup>2+</sup> and Au<sup>+</sup> over broad concn. ranges produced nonlinear ECL response curves. Despite low-level ECL, sensitivities in the ppm range for Au<sup>+</sup>, Cu<sup>2+</sup>, and their resp. DAT isomers were achieved. Time dependence was obsd. for some of the ECL reactions, including the Ru(III)-bipyridine model system, in which the ECL intensity grew markedly over several hours. No ECL enhancements over background were obsd. with two dinitrotoluene isomers or an aminonitrotoluene screened against the same set of 32 metals. This novel ECL approach may have applications in the detn. of some aminoaroms. from degrdn. of explosives (e.g., TNT) as well as detection and quantitation of various transition metals in industrial wastewater streams and groundwater supplies. In terms of fundamental science, the present data are probably of interest as an example of size-dependent mol. recognition of metal ions which can be detected by ECL.

ST electrochemiluminescence detn diaminotoluene isomer; gold reagent  
diaminotoluene detn electrochemiluminescence; copper reagent  
diaminotoluene detn electrochemiluminescence

IT Chemiluminescence spectroscopy

(electro-; an electrochemiluminescence assay based on the interaction  
of diaminotoluene isomers with gold(I) and copper(II) ions)

IT 95-80-7, 2,4-Diaminotoluene 496-72-0, 3,4-Diaminotoluene

RL: ANT (Analyte); ANST (Analytical study)

(an electrochemiluminescence assay based on the interaction of  
diaminotoluene isomers with gold(I) and copper(II) ions)

IT 7440-50-8, Copper, uses 7440-57-5, Gold, uses

RL: ARG (Analytical reagent use); ANST (Analytical study); USES (Uses)

(an electrochemiluminescence assay based on the interaction of  
diaminotoluene isomers with gold(I) and copper(II) ions)



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Volume 56, Issue 3, July 1997, Pages 305-314

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Based on the Interaction of Diaminotoluene Isomers with  
Gold(I) and Copper(II) Ions**John G. Bruno <sup>a,1</sup> and Jimmy C. Cornette <sup>b</sup><sup>a</sup> Applied Research Associates, 139 Barnes Drive, Suite 2, Tyndall Air Force Base, Florida, 32403<sup>b</sup> U.S. Air Force, Armstrong Laboratory, Environics Directorate, 139 Barnes Drive, Suite 2, Tyndall Air Force Base, Florida, 32403

Received 12 April 1996; accepted 19 July 1996. Available online 15 April 2002.

**Abstract**

A novel assay for the determination of 2,4- and 3,4-diaminotoluene (DAT) isomers based on the low-level electrochemiluminescence (ECL) reaction of these molecules with the group IB transition metal ions  $\text{Au}^+$  and  $\text{Cu}^{+2}$ , respectively, in aqueous solution is described. DAT isomers were screened for ECL against a repertoire of 32 metals, including metal ions such as  $\text{Cu}^{+2}$ ,  $\text{Eu}^{+3}$ ,  $\text{Mg}^{+2}$ ,  $\text{Ru}^{+3}$ , and  $\text{Tb}^{+3}$  associated with other known ECL complexes, at 1:3 added metal ion:ligand molar ratios. The 1:3 molar ratio presumed tris-bidentate octahedral metal coordination complex formation, which generally yielded optimal ECL intensity. The apparent specificity of  $\text{Au}^+$  for 2,4-DAT and  $\text{Cu}^{+2}$  for 3,4-DAT, as indicated by ECL measurements, may be partly based on ionic size as  $\text{Au}^+$  has nearly twice the ionic diameter of  $\text{Cu}^{+2}$  and thus may form a coordination complex with the *meta*, but not the *ortho* DAT. Other DAT isomers were screened and exhibited mildly enhanced ECL with various metal ions, including group IB transition metal ions, but these ECL enhancements were not statistically significant. In some cases, titration of DAT ligands with  $\text{Cu}^{+2}$  and  $\text{Au}^+$  over broad concentration ranges produced nonlinear ECL response curves. Despite low-level ECL, sensitivities in the ppm range for  $\text{Au}^+$ ,  $\text{Cu}^{+2}$ , and their respective DAT isomers were achieved. Time dependence was observed

tunichrome complexes, properties 7439-93-2D, Lithium, tunichrome complexes, properties 7439-97-6D, Mercury, tunichrome complexes, properties 7440-09-7D, Potassium, tunichrome complexes, properties 7440-22-4D, Silver, tunichrome complexes, properties 7440-38-2D, Arsenic, tunichrome complexes, properties 7440-39-3D, Barium, tunichrome complexes, properties 7440-43-9D, Cadmium, tunichrome complexes, properties 7440-47-3D, Chromium, tunichrome complexes, properties 7440-48-4D, Cobalt, tunichrome complexes, properties 7440-50-8D, Copper, tunichrome complexes, properties 7440-53-1D, Europium, tunichrome complexes, properties 7440-55-3D, Gallium, tunichrome complexes, properties 7440-57-5D, Gold, tunichrome complexes, properties 7440-70-2D, Calcium, tunichrome complexes, properties 119046-42-3, 2,3,4-Tunichrome 119080-89-6, 3,4,5-Tunichrome  
RL: PEP (Physical, engineering or chemical process); PRP (Properties); PROC (Process)

(electrochemiluminescence from tunicate, tunichrome-metal complexes and other biol. samples)

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<sup>1</sup> To whom correspondence should be addressed.

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doi:10.1006/mchj.1996.1405 ? Cite or link using doi

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# An Electrochemiluminescence Assay Based on the Interaction of Diaminotoluene Isomers with Gold(I) and Copper(II) Ions

John G. Bruno <sup>a,1</sup> and Jimmy C. Cornette <sup>b</sup><sup>a</sup> Applied Research Associates, 139 Barnes Drive, Suite 2, Tyndall Air Force Base, Florida, 32403<sup>b</sup> U.S. Air Force, Armstrong Laboratory, Environics Directorate, 139 Barnes Drive, Suite 2, Tyndall Air Force Base, Florida, 32403

Received 12 April 1996; accepted 19 July 1996. Available online 15 April 2002.

**Abstract**

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